

Sensitivity test of *staphylococcus aureus* against extract *tinospora crisper*

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Abstract

A bacterium such as *Staphylococcus aureus* (*S. aureus*) produces a kind of toxic protein which can disrupt intestinal wall. Livestock reacts to these toxins by pumping lots of water into the intestine in order to rinse or flush these toxins. As a result, the livestock have diarrhea as a body response to remove the toxin in the digestive system. In the presence of these problems, farmers take a measure such as using antibiotics freely. Among farmers, antibiotics are often used freely without knowing the indication and the appropriate dose for the treatment of livestock diseases. It is caused due to the antibiotics easily obtained in animal drugstores which leading to bacterium resistance to these antibiotics. Another alternative done by the farmers is by using plants as medicinal herb such as *Tinospora crispa* (*T. crispa*). The purpose of this research is to examine *S. aureus* bacterium sensitivity against *T. crispa* extract. The study was conducted using *T. crispa* extract with a concentration of 10%, 30%, 50% and 70%. A positive control using Levofloxacin. The result of the research shows that *T. crispa* extract with the concentration of 10%, 30%, 50% and 70% are more sensitive than Levofloxacin. The content found in *T. crispa* such as alkaloids, flavonoids, and saponins are antibacterial which has a satisfying effect to kill *S. aureus*.

Keywords : Antibacterial *Tinospora crispa*, *Staphylococcus aureus*

1 Introduction

S. aureus is one Gram positive cocci-shaped bacteria that is pathogenic to humans. It causes 70% of cases of nosocomial infections. *S. aureus* can cause infections of the skin and soft tissue invasive such as pneumonia, osteomyelitis, meningitis and endocarditis. Antibiotics among breeders are often used freely without knowing the indication and the appropriate dose for the treatment of livestock diseases. Such uncontrolled implementation could endanger consumers. For instance, in some cases, farmers do not know that the incorrect dose could leave residues in meat, milk and eggs. Another example is to mix milk from cows that are getting treatment with antibiotics, with milk from healthy cows. As a result, the milk will be unsafe for consumption because of contamination by bacteria and antibiotic residues [1]. In Indonesia, the use of antibiotics using tetracycline, penicillin, chloramphenicol, erythromycin and streptomycin is quite dominant. Whereas in some other countries, the use of these antibiotics has reached excessive levels and many of them are even used inappropriately. The development of antibiotics bacterial resistance is strongly influenced by the intensity of exposure in a regional area, where uncontrolled use of antibiotics tends to increase the resistance of bacteria which is previously sensitive.

T. crispa is an herbaceous vine which extensively grows in tropical and subtropical regions of Southeast Asia [2]. Farmers in the regional area are using herbs such as *Tinospora crispa* to treat animal diseases such as ulcers, fever, and also even worms [3]. *Tinospora crispa* also contains soft resin, starch, glycosides, picroretosid, bitter substances like picroretin, Harsha, alkaloid berberine and palmatin. The *Tinospora crispa*'s roots also contain alkaloids berberine and kolumbin. Its leaves and stems contain alkaloids, saponins and tannins and the trunk contains flavonoids, alkaloids, saponins [4].

Based on the above, we formulate the problem as follows:

- How much contamination of *Staphylococcus aureus* are there in the dairy livestock products?
- Will the *Tinospora crispa*'s extract be effective in killing the *Staphylococcus aureus*?
- At which level the extract's concentration be effective against *Staphylococcus aureus*?

1.1 Research objectives

- To analyze the bacterial contamination of pathogenic *Staphylococcus aureus* in dairy livestock products.
- To identify the *Staphylococcus aureus* genotype, known as the cause of human zoonotic.

- To analyze the resistance of *S. aureus* against *Tinospora crispa*.
- To provide contributions for the purpose of public health to reduce the spread of zoonotic disease caused by *Staphylococcus aureus*, which is spread through food ingredients from cattle.

1.2 Methodology

Cow milk samples were taken from dairy farms Enrekang districts in South Sulawesi. The number of samples were taken using Martin's Formula [5]. N = 280

• Phase One: isolation and identification

For the *S. aureus* identification with Gram stain, it would form a cluster of round shape like a string of grapes and are Gram-positive. The catalase positif, biochemical test, *S. aureus* with media Mannitol Salt Agar, the bacteria *S. aureus*, will form yellow colonies [6]. The specific *S. aureus* was tested with the antibiotic Novobiocin that will look sensitive.

- **Phase Two - PCR:** To verify the *S. aureus*, we use PCR amplification using the DNA thermal Cycler. We made the 2 % Gel Agarosa by dissolving 2 g agarose (BioRad) within a 100 ml 10 Tris borate EDTA (100 g Tris base, 27.5 g asam borat, 20 ml 0.5 M EDTA pH 8.0 within a 1 liter water). Afterward, we added by 1 µl ethidium bromida (0.2 ug/ml), which then put in into an Electrophoresis tank with TBE 0.5x. Primer Sea

F TTGGAAACGGTTAAAACGAA

R GAACCTTCCCATCAAAAACA

Ladder 100 bp solution.

- **Phase three: Antimicrobials test.** Sensitivity Indicators is identified by the formation of a clear zone around the paper disk, and then measured the diameter of clear zone formed and adapted to the standards of the products that disk.

2 Result and Discussion

The number of samples 280, 107 samples positive California Mastitis Test (CMT).

2.1 Tests against *S. aureus*

CMT positive milk samples were inoculated at MSA, marked with yellow colonies. *S. aureus* on this medium can ferment mannitol and produce acid, as well as changes color to yellow media. In addition to *S. aureus*, bacteria *S. saprophytic* colony also has a yellow color. When inoculated on specific media, some pathogenic

microorganisms will produce color in her colonies and can be used for identification of isolates [7]. As the two bacteria have the same color, additionally, we diagnosed *S. aureus* using antibiotic novobiocin where *S. aureus* is sensitive to novobiocin, different to *S. saprophytic* is resistant to novobiocin.

Of the 107 samples with positive CMT there were 42 positive samples of *S. aureus* in MSA test and 35 samples with novobiocin test

Catalase test is conducted to observe the bacteria's ability to produce the enzyme catalase. *Staphylococcus aureus* bacteria are able to produce the enzyme catalase. This test is performed using 3% H₂O₂. Catalase enzyme from bacteria was able to break down hydrogen peroxide (H₂O₂), so that when a colony of bacteria mixed with H₂O₂ 3%, will be formed the gas bubbles.

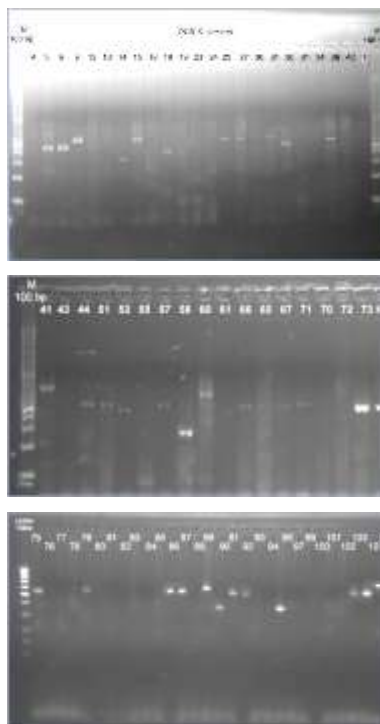


Fig 1 : PCR Test against *S. aureus*

PCR *S. aureus* Examination

From 107 positive of CMT milk samples, 42 were positive using the MSA. *S. aureus* on this medium was able to create fermentation on mannitol and produce acid which caused the color change to yellow. On this testing method, it is not only *S. aureus* was turning yellow but also the *S. saprophytic* as well.

To confirm *S.aureus*, it was further tested with novobiocin. There were 35 positive samples out of 42 positive MSA, PCR test was further carried out on those positive samples. Evidently 35 positive samples obtained the same result using this novobiocin. Of the 107 CMT positive milk samples, it contained 35 samples were positive *S.aureus*. by PCR test

Bacteria in the milk can be caused by mastitis in the tool or storage during transportation or during processing time [8] According to [9], pathogenic bacteria that can be found in milk includes *Staphylococcus aureus* (*S. aureus*).

Table 1 The sensitivity of antibiotics to *S.aureus*

1.	Ampicillin	Inhibit bacterial cell wall synthesis	42.3 (S)
12.	Imipenem	Inhibit bacterial cell wall synthesis	54.1 (S)
19.	Tetracycline	Inhibit bacterial protein synthesis	34.55 (S)
24.	Levofloxacin	Inhibit bacterial nucleic acid synthesis	32.6 (S)
27.	Novobiocin	Inhibit bacterial nucleic acid synthesis	42.3 (S)
			<i>S.aureus</i>

Source (10)

From various antibiotic tests as above, it is apparent that Levofloxacin has the least potency

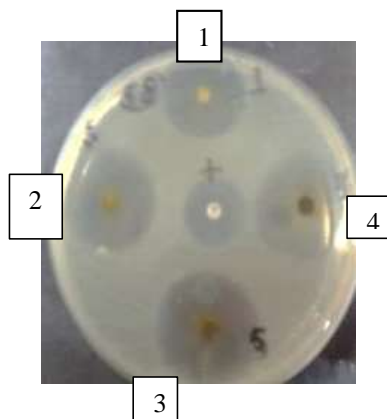


Fig 2 : Sensitivity test *S.aureus* and extrax *Tinospora crispa* 10% (1), 30% (2), 50% (3), 70% (4), and control (+)

On sensitivity test on *Tinospora crispa* with concentrations of 10, 30, 50 and 70% with a positive control of Levofloxacin, we notice an inhibition zone 32. mm with a description of the sensitive. This is consistent compare with control positive Levofloxacin (32.6.mm) means that *T.crispa* sensitive to *S.aureus*.

Based on the test results of *Tinospora crispa* activity against *Staphylococcus aureus*, we obtained that inhibitory zone diameter at concentrations *Tinospora crispa* 10%, (35 mm) has a strong antibacterial response inhibition. Moreover, the concentration of 30%, (42 mm) has a strong antibacterial inhibitory response, concentration of 50%, (44 mm) including strong categories, and the greatest inhibition zone at a concentration of 70%, (47 mm). The result of the classification criteria shows that the bacteria inhibition quite strong category.

Bacteria sensitivity test is a method for determining the bacteria resistance to antibacterial agents. In this study, we used natural materials *Tinospora crispa* for their antibacterial ability to reduce the growth or kill bacteria *S. aureus*. Testing *Tinospora crispa* against *S. aureus* compared with Levofloxacin as control positif (+). In antibiotic Levofloxacin (LEV) obtained inhibition zone 32,6 mm with a caption sensitive. Levofloxacin works by inhibiting bacterial DNA duplication, thus preventing its development. Based on these results, Levofloxacin antibiotic were used for the treatment of diseases caused by bacterial infections *Staphylococcus aureus*. The widening the diameter of inhibition zone formed by these bacteria more sensitive [11]. From the results obtained it turned *T.crispa* 10% more sensitive than Levofloxacin (Table 2)

Table 2 Test Results *T. crispa* and antibiotics against *S. aureus*

Concentration	mm
	<i>S.aureus</i>
<i>T.crispa</i> 10%	35
<i>T.crispa</i> 30%	42
<i>T.crispa</i> 50%	44
<i>T.crispa</i> 70%	47
Levofloxacin 5	32

3 Conclusion

Milk dairy cattle are tested against *S. aureus* were contaminated with *S. aureus* 35 samples of 107 positive CMT cattle (32.71 %), *T.crispa* sensifitas against

S.aureus sensitive to *T.crispa* 10% 30%, 50% 70% ,35 mm, 42 mm ,44 m and 47 mm compared to Levofloxacin (32 mm) *T.crispa* of these results can be used to inhibit and kill bacteria *S.aureus*

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References

- [1] Murdiati, T.B. 2004. Advanced and Management Chemical Use in Farm Practices. Proc. of the 4th Asian Conference on the Food, and Nutrition Safety Organized by ILSI, FAO and Bogor Agricultural University. pp. 86 – 96.
- [2] Pathak A. K., Jain D. C., Sharma R. P. (1995). Chemistry and biological activities of the genera *Tinospora*. *Pharm. Biol.* 33, 277–287.
- [3] Apriani SN. 2013. Senyawa Flavanoid yang Bersifat Antibakteri dari Akway (*Drimys beccariana*. Gibbs). Fakultas Matematika dan Ilmu Pengetahuan Alam. Universitas Negeri Papua.
- [4] Kresnady, B., 2003, Khasiat dan Manfaat *Tinospora crispa* si-Pahit yang Menyembuhkan, PT Agromedia Pustaka, Jakarta.
- [5] Martin SW, Meek AH, Willeberg P.1987. Veterinary Epidemiology. USA:Iowa State University Press
- [6] Tortora,G.J.,B.R.Funkw.,C.L.Case. 1995.Microbiology. An Introduction.The Benyamin/Cumming Publishing Com.Ins.Sydney
- [7] Acharya Aastha, Aastha Poudel, Ajay Kumar Sah, Divya Maharjan, Simran Tibrewal and Pappu Kumar Mandal. 2016. Isolation and Identification of Bacteria from Meat Processing Units of Kathmandu Valley
- [8] Lund, P.; Weisbjerg, M. R. ; Hvelplund, T., 2006. Passage kinetics of fibre in dairy cows . obtained from duodenal and faecal ytterbium excretion - Effect of of forage type. . *Feed Sci. Technol.*, 128 (3-4): 229-252
- [9] Fardiaz, S. 1993. Analisis Mikrobiologi Pangan. PT. Raja Grafindo Persada, Jakarta.
- [10] Lucia Winata Muslimin, Abdul Wahid Jamaluddin, Ressa Dwiyanti, , Mochammad Hatta, 2015.American Journal of Biomedical and Life Sciences 2014; 2(6): 163-166. Published online January 07, 2015
- [11] Gaman, P.M dan K.B. Sherrington, 1992.Pengantar Ilmu Pangan, Nutrisi,dan Mikrobiologi. Terjemahan M. Gardjito, dkk. UGM-Press, Yogyakarta